

Claims:

1. A method of producing a recombinant polypeptide, comprising;
expressing a glycosylated recombinant polypeptide in the
5 plastid of a plant cell.
2. A method of producing a recombinant polypeptide comprising;
expressing in a plant cell a nucleic acid encoding a fusion
polypeptide which comprises said recombinant polypeptide, an ER
10 signal sequence and one or more ER-plastid targeting sequences.
3. A method according to claim 2 wherein said plant ER signal
sequence is from an ER processed plastid polypeptide.
- 15 4. A method according to claim 2 or claim 3 wherein the one or
more ER-plastid targeting sequences comprise at least 10 contiguous
amino acids from an ER-processed plastid polypeptide.
5. A method according to claim 4 wherein the at least 10
20 contiguous amino acids comprise two or more contiguous basic
residues.
6. A method according to any one of claims 2 to 5 wherein the one
or more ER-plastid targeting sequences are comprised within an ER-
25 processed plastid polypeptide.
7. A method according to claim 6 wherein the ER-processed plastid
polypeptide has a sequence listed in Table 1.
- 30 8. A method according to claim 6 wherein the ER-processed
plastid-localised polypeptide is a CAH1 polypeptide.
9. A method according to any one of claims 2 to 8 comprising
cleaving said expressed fusion polypeptide to generate said
35 recombinant polypeptide.

10. A method according to claim 9 wherein the expressed fusion polypeptide comprises one or more cleavable linker sequences, said recombinant polypeptide being generated by cleavage of said one or more linker sequences.

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11. A method according to claim 10 wherein said one or more linker sequences are cleaved within said plastid by a heterologous endoprotease to generate said recombinant polypeptide.

10 12. A method according to claim 10 wherein said one or more linker sequences are cleaved within said plastid by an endogenous plastid endoprotease to generate said recombinant polypeptide.

15 13. A method according to any one of the preceding claims comprising isolating and/or purifying said recombinant polypeptide from a plastid of said cell.

20 14. A method according to any one of claims 1 to 10 comprising isolating and/or purifying said expressed fusion polypeptide from a plastid of said cell prior to cleavage to generate said recombinant polypeptide.

25 15. A method according to any one of the preceding claims wherein the recombinant polypeptide comprises one or more glycosylation sites.

16. A method according to claim 15 comprising determining the glycosylation of the expressed recombinant polypeptide.

30 17. A method according to any one of the preceding claims wherein said plastid is a chloroplast.

35 18. A nucleic acid construct comprising;
a nucleotide sequence which encodes an ER signal sequence,
one or more ER-plastid targeting sequences, and;

one or more restriction endonuclease sites for insertion of a nucleotide coding sequence capable of expressing a recombinant polypeptide fused to said ER signal and ER-plastid targeting sequences.

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19. A nucleic acid construct according to claim 18 comprising;
a nucleotide coding sequence capable of expressing a recombinant polypeptide fused to said ER signal and ER-plastid targeting sequences,

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said coding sequence being inserted in the one or more restriction endonuclease sites.

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20. A nucleic acid construct according to claim 18 or claim 19 wherein the nucleotide sequence further encodes one or more cleavable linker sequences,

said recombinant polypeptide being generated by cleavage of said one or more linker sequences.

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21. A nucleic acid construct according to any one of claims 18 to 20 wherein said ER signal sequence is from an ER-processed plastid polypeptide.

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22. A nucleic acid construct according to any one of claims 18 to 21 wherein the one or more ER-plastid targeting sequences comprise at least 10 contiguous amino acids from an ER-processed plastid polypeptide.

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23. A nucleic acid construct according to any one of claims 18 to 22 wherein the one or more ER-plastid targeting sequences comprise two or more contiguous basic residues.

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24. A nucleic acid construct according to any one of claims 18 to 23 wherein the ER signal sequence and one or more ER-plastid targeting sequences are comprised within an ER-processed plastid polypeptide sequence.

25. A nucleic acid construct according to any one of claims 18 to 24 wherein the ER-processed plastid localised polypeptide sequence is a sequence listed in Table 1.
- 5 26. A nucleic acid construct according to any one of claims 18 to 24 wherein the ER-processed plastid-localised polypeptide sequence is a CAH1 polypeptide.
- 10 27. A nucleic acid construct according to any one of claims 18 to 26 wherein said plastid is a chloroplast.
- 15 28. A nucleic acid vector suitable for transformation of a plant cell and comprising a nucleic acid construct according to any one of claims 18 to 27.
29. A host cell comprising a nucleic acid construct according to any one of claims 18 to 27 or a vector according to claim 28.
- 20 30. A host cell according to claim 29 having said nucleic acid construct or vector within its genome.
31. A host cell according to claim 29 or claim 30 which is a plant cell.
- 25 32. A plant cell according to claim 31 which comprises nucleic acid encoding one or more mammalian glycosyltransferases.
- 30 33. A plant cell according to claim 31 or claim 32 which is deficient in one or more plant specific glycosyltransferases.
34. A plant cell according to any one of claims 31 to 33 which is comprised in a plant, a plant part or a plant propagule, or extract or derivative of a plant.

35. A method of producing a cell according to any one of claims 29 to 33 the method comprising incorporating said nucleic acid construct or vector into the cell by means of transformation.

5 36. A method according to claim 35 which comprises combining the nucleic acid with the cell genome nucleic acid such that it is stably incorporated therein.

10 37. A method according to claim 35 or claim 36 which comprises regenerating a plant from one or more transformed cells.

15 38. A method according to claim 37 comprising sexually or asexually propagating or growing off-spring or a descendant of the plant regenerated from said plant cell.

39. A plant comprising a cell according to any one of claims 31 to 33.

20 40. A method of producing a plant according to claim 36, the method comprising incorporating a nucleic acid construct according to any one of claims 18 to 27 into a plant cell and regenerating a plant from said plant cell.

25 41. Use of a nucleic acid according to any one of claims 18 to 27, a vector according to claim 28, a cell according to any one of claims 29 to 33 or a plant according to claim 39 in a method of producing a recombinant polypeptide.